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Behavioural indicators of pain in crustacean decapods

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Summary. Whether invertebrates are able or not to experience pain is a highly controversial issue. An operative way to solve such a controversy might be to investigate their responses to potentially noxious stimuli and to collect evidence of their behavioural complexities as proxies of cognitive capacities. The principle of argument-by-analogy can be then applied to these data: the behaviour displayed by invertebrates is compared with that of “higher” animals, its similarity denoting the former’s capacity to have analogous experiences. Here, the author discusses some examples, extracted from the literature on crustacean decapods, that pinpoint their nature of “sentient” animals. This review, however, also shows that research is still scanty in the field. The studies that examine the potential links between stress responses and pain experience are few, and the several papers that help elucidate cognitive abilities in decapods have been limited to a few taxa and are not specifically directed to the question of “sentience”. On the contrary, also in the light of the expected revision of the current EU legislation in the matter, more scientific efforts should be expended on exploring the issue of pain experience in invertebrates.

Key words: pain, crustacean decapods, behaviour, animal welfare.

Riassunto (*Indicatori comportamentali di “sofferenza” nei crostacei decapodi*). Il tema della “sofferenza” da parte degli invertebrati è assai controverso. Da un punto di vista operativo, il problema potrebbe essere affrontato analizzando le risposte nei confronti di stimoli potenzialmente dolorosi e la complessità del comportamento come indice delle loro capacità cognitive. Ai dati ottenuti è possibile applicare l’argomentazione per analogia. Il comportamento osservato è confrontato con quello degli animali “superiori”, dove le similarità eventualmente riscontrate potrebbero indicare la capacità degli invertebrati a possedere esperienze analoghe a quelle dei vertebrati. In questa revisione, si discuteranno alcuni esempi tratti dalla letteratura dei crostacei decapodi che potrebbero suggerire la loro natura di animali “senzienti”. Si osserverà, comunque, che la ricerca in questo settore è ancora limitata. Gli studi che esaminano la relazione tra risposte comportamentali e stimoli dolorosi sono scarse e le varie pubblicazioni che evidenziano comportamenti complessi nei decapodi riguardano un esiguo numero di specie. Al contrario, anche in risposta all’attesa revisione della legislazione europea in tema di benessere animale, appare necessario rivolgere maggiore interesse scientifico al problema della sofferenza negli invertebrati.

Parole chiave: sofferenza, crostacei decapodi, comportamento, benessere animale.

INTRODUCTION

The capacity of an animal to feel pain has a clear adaptive value: by enhancing the likelihood of an organism to stay alive long enough to produce offspring, pain contributes to increase the fitness of the experiencing animal [1, 2]. As a consequence, this capacity should have had an early appearance during evolution and should be widespread in the animal kingdom [3]. Against this reasoning, however, the recognition of its occurrence in invertebrates is still highly controversial [4].

In large part, such a controversy may be the result of the very nature of suffering: pain does not imply only unconscious reflex responses that assist in withdrawing from noxious, tissue-damaging stimuli [5], but it also includes awareness of such stimuli with

the intervention of the conscious part of the “brain”, which is most difficult to prove in an animal. To make things worse, in the definition of human pain (an “unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage”; International Association for the Study of Pain, November 2007) [6], the stress is given to the “emotional experience” of an individual, which is a private experience. It is obviously impossible to truly know whether an animal has such an experience. Therefore, this concept is usually excluded from the definitions of pain in animals, as those provided by Zimmerman [7] –“an aversive sensory experience caused by actual or potential injury that elicits protective motor and vegetative reactions, results in learned avoidance and

may modify species-specific behaviour, including social behaviour”—or Broom [8]—“an aversive sensation and feeling associated with actual or potential tissue damage”.

The difficulties encountered when tackling the issue of pain in invertebrates also arise from the attitude that the general public has always shown towards this taxon. Arthropods in particular generate fear and aversion to many people [9]. Additionally, they are also viewed as good “replacement” alternatives for vertebrates in biomedical research and in toxicity testing [10]. Invertebrates are in general assumed to be insentient, or at least less sentient than vertebrate animals. Two issues have been raised to support this belief [11–13]. The first is the occasionally noted absence of behavioural responses in such conditions that we would expect to elicit great responsiveness from vertebrates: for instance, some insects will continue to feed whilst being eaten by predators or, as shown in the case of the male praying mantis, by their sexual partners [11]. The second is the lack of complexity in the invertebrates’ nervous system, an issue based on the widespread assumption that the best estimate of nervous system sophistication is its nearness to humans [14]. The idea that invertebrates are intrinsically unable to suffer is fostered by some records of species exhibiting largely pre-programmed behaviour patterns and quantitatively differing in their physiology with “higher” taxa [11].

ASSESSING PAIN

An operative way to deal with pain in invertebrates, as well as in other non-human animals, has been to take indirect measurements of behavioural and physiological responses to potentially noxious and distressing stimuli, and then assess the evidence, as is routinely done in welfare studies [1, 14–17]. Other indicators of pain experience by invertebrates have been identified [18], such as having a suitable nervous system, showing trade-offs between stimulus avoidance and other motivational requirements, having opioid receptors, reducing responses to noxious stimuli by analgesics and local anaesthetics, and having cognitive capacities. In this field, the principle of argument-by-analogy is usually applied [2]: the behaviour displayed by invertebrates is compared with that of “higher” animals, its similarity denoting the former’s capacity to have analogous experiences. Work in fishes [5] has shown how powerful a case can be made for animal sentience on the basis of an argument by analogy [19]. Following the way opened by researchers in mollusc cephalopods [9, 20], recent studies in crustacean decapods have started to make a case for sentience in invertebrates [4, 21] as well.

A review about the central nervous system in crustacean decapods, opioid receptors, and the effects of local anaesthetics or analgesics is available in Elwood *et al.* [22]. Below the author will extend

the discussion to other examples of decapods’ behaviour that might be indicative of non-reflexive responses to noxious stimuli and of relatively complex cognitive capacities in this taxon.

RESPONSES TO NOXIOUS STIMULI

The responses by decapods to minimize noxious stimuli have been the focus of a few studies only. The first regards the glass prawn, *Palaemon elegans* [21]. When sodium hydroxide or acetic acid solution was applied to one antenna, prawns first showed a reflex tail-flick response, followed by the prolonged grooming of the antenna and rubbing it against the side of the tank. Both these activities were more intense than when water was applied, were directed to the treated antenna alone (suggesting the prawn’s awareness of the specific location of the noxious stimulus), and were inhibited by the anaesthetic benzocaine.

In a similar experiment, the hermit crab *Pagurus bernhardus* was subject to small electric shocks. Significantly more shocked animals evacuated their shells than control hermit crabs, indicating the aversive nature of the stimulus [4]. However, few hermit crabs evacuated the occupied shell when this belonged to a preferred species, but did so when offered a new high-quality shell. This denotes a clear motivational trade-off that is also expression of a relatively refined cognitive capacity by this taxon. Responses analogous to those shown by prawns and hermit crabs have been often observed in vertebrates [23] and interpreted as indices of their awareness of the painful stimulus [5, 24].

A second set of evidence comes from leg autotomy. This behaviour, often occurring in crustacean decapods, can be mediated by noxious stimuli, as for instance shown in the crab *Carcinus spp.* placed on a hot plate [25] or exposed to a small electric shock [22]. Heat and electric shocks cause reactions in vertebrates that are indicative of pain [e.g. 5, 26]; based on the argument-by-analogy, the conclusion might be that autotomy is elicited in decapods by a similar experience.

A more refined response to noxious stimuli is “avoidance learning”. The crayfish *Procambarus clarkii* and the crab *Chasmagnathus granulatus* were found to associate an electric shock with the turning on of a light or with the occupancy of the light compartment of the maintenance aquarium, respectively [27, 28]. They quickly learned to respond to these associations by walking to a safe area in which the shock was not delivered (in the case of the crayfish) or by refraining from entering the light compartment (in the case of the crab). Memory of the association was retained for a relatively long time, up to 24 h after a multi-trial training test in crabs [29]. Interestingly, despite repeated pairings of light and shock, crayfish did not learn to avoid the shock by tail-flipping away in response to light [27]. This inability to form some associations agrees with what is usually observed in mammalian avoidance learning: Bolles [30], for in-

stance, found that rats had difficulty in learning to press a lever to avoid electric shock, despite the fact that the same lever-press response could be easily acquired with positive reinforcement.

COGNITIVE CAPACITIES

Definition

Most indicators of consciousness listed in the literature, such as the possession of language, the capacity of metacognition and the ability to make first-person reports of the contents of one's thought, are essentially anthropocentric [see 31 for a discussion]. Based on the idea that an evolutionary continuity exists and that non-human animals might have a simpler ability ("primary consciousness"), the investigation of this capacity has been recently extended to some animal taxa, including cephalopods [31, 32]. Evidence focuses on the complexity and flexibility of the behaviour of the group of animals in question and on the possibility that a general "global workspace" with an attentional spotlight [33] oversees and organizes evaluation of sensory processing. Operative definitions have been set forth, in which cognitive capacities are interpreted as the result of an animal's faculty of processing the information gathered through sensory organs from its internal and external environment, and of storing and retrieving it for optimal adaptation to its current physical and social environments [34].

A number of studies, although not specifically directed to the issue of pain, shows the ability of crustacean decapods to display such a rich behavioural repertoire that, if exhibited by vertebrates, would have been considered to be indicative of higher mental faculties [2]. Again, the underlying rationale is that animals that possess such behaviours are sentient and may experience pain [14, 19, 35]. Understandably, complexity in behaviour does not indicate consciousness but it may set out the basis for it [31].

Discrimination abilities

Decapods rely on a combination of sophisticated sensory systems to identify food, mates, refuges, and predators; they choose among alternatives, and make adaptive decisions. There are two well-studied cases that exemplify this taxon's discrimination abilities. The first regards mate choice in the crayfish *P. clarkii*. When analysed in a binary-choice test paradigm, females of this species were found to select mates with large body size [36]. This is consistent with the traditional ideas of sexual selection theories: large males are relatively more fertile with respect to smaller individuals and, being dominant in intra-sexual competition, offer the females with high-quality resources, such as breeding burrows. However, the ability to discriminate between small and large males, although being widespread in invertebrates, seems to be extremely refined in *P. clarkii*. The females select the larger male relying not only

on sight but also on odour [37]. Visual and chemical stimuli act as non-redundant signals by providing complementary information that allows the females to discriminate quicker the higher-quality mate in the crowded social context experienced during the mating period [38]. Discrimination is so accurate that, if forced to copulate with small males, females "decide" to invest relatively less in terms of the size of the spawned eggs, being able to adjust the quantity of egg deutoplasm as a function of the mate body size [39]. An additional mechanism allows for the identification of a potential mate of higher quality when a female is offered with two males of the same size: the female eavesdrops on the two fighting crayfish and then selects the winner [40]. This ability to discriminate the quality of two males based on social eavesdropping relies again on the combination of sight and smell; interestingly, the winner is selected only after the female has observed and smelled that individual male and not a generic one, thus denoting the intervention of a refined form of individual recognition [41].

A second abundantly studied example of discrimination abilities in decapods is shell selection in hermit crabs. Gastropod shells of appropriate size and shape are vital for most hermit crab species, being essential for their survival, growth, and reproduction. The shell protects the soft uncalcified abdomen from attack by predators and also plays a role in buffering against environmental extremes. However, empty shells (hermit crabs are unable to directly prey on living snails) are in acutely short supply in the habitat so that a number of refined behavioural mechanisms evolved to find them [42]. Several studies have shown that hermit crabs of different species show an outstanding ability to finely discriminate the relative quality of the offered shell by gathering, manipulating, and integrating an array of information. For instance, they are able to integrate information about the offered shell with information about the shell they currently occupy before deciding which is the better of the two [43]. In making decisions, hermit crabs use visual and tactile stimuli that inform of the shape and size of the new shell, and integrate them with additional proprioceptive stimuli gathered after having moved in it [44]. They often fight with con- or heterospecifics over the possession of shells: the attacker tries to evict the defender from its shell but the progress with the attack and the outcome of the fight depend on a number of factors including the relative size of the crabs and the pre-fight displays used [45], the perceived value of the attacker's shell [46, 47], the quality of the defender's shell [48, 49], and the quality of previous shells [50]. For instance, when subject to an experimental worsening in the quality of their shell, hermit crabs are more aggressive than those subject to an improvement; the former were even more aggressive than the individuals that, before manipulation, occupied a bad quality shell [50]. Besides, the decisions made by the defender about whether or not to resist is based on

the assessed vigour of the attacker [51] whereas the strategic choices made by the attacker derive from the integrated information about the strength of the opponent and the quality of the two shells at stake [52]. Finally, the attacker constantly monitors changes in its physiological state and adapts its behaviour accordingly [53].

Complex learning

The ability to establish complex associations between two (or more) stimuli, to respond to them in an adaptive fashion, and to retain their memory for a relatively long time is widespread among decapods and is expressed in several biological contexts with clear adaptive values. One obvious context is feeding. For instance, the spiny lobster *Panulirus argus* is able to associate dangers with the odour of a given food and subsequently avoids searching for these odours [54]. Differential conditioning shows that individuals of this species learn to discriminate chemical mixtures that differ only in the ratios of their components. They can remember these odours and perform discrimination tasks for several days [55].

The most refined expression of associative learning in decapods seems to be the one leading to the identification of conspecifics. A form of social recognition has been described in crayfish, lobsters, and hermit crabs [56–58] and seems to be mostly mediated by pheromones released in the urine [59–61]. Illustrative in this respect is the ability of “individual recognition” recently demonstrated in the hermit crab *Pagurus longicarpus*. A series of laboratory experiments had shown that hermit crabs of this species can discriminate between their own odours and the odours of other individuals; they are also able to recognise the odours of at least two conspecifics they had previously met and with whom they either won or lost fights [62, 63]. This recognition ability seems to be a particularly sophisticated task. First, it can be achieved after a brief (less than 30 min) exposure to the stimulus animal but it lasts for a relatively long time (about five days) [64]. Second, hermit crabs do not require specific training but show evidence of recognition without external, experimenter-provided reinforcement or punishment. Third, recognition seems to be the result of the established association between the odour of a known conspecific and the quality of the shell it occupies.

This latter evidence might suggest that the recognising hermit crab uses the individual odour of the conspecific as a label of shell quality; if this label indicates high shell quality, their detection evokes an intense shell investigation towards the offered shell; otherwise, shell investigation is scarce or absent. The association between individual odour and shell quality is also highly plastic: once an individual crab has been switched to a shell of a higher or lower quality, subsequent responses to the offered shell appear consistent with the changed association. In summary, hermit crabs seem to associate a type of information emitted by the social partner (e.g. urine-

borne pheromones) with some experience with it that offers information about the quality of its shell (e.g. exploration of its shell), and retain memory of this association for a relatively long time, being however ready to adjust their behaviour when shell quality changes. In other words, they seem to have a “concept” of other individuals and behave accordingly. The adaptive value of this ability possibly lies in the advantage that an individual may have from a quicker assessment of the quality of the shell occupied by a conspecific when a decision is to be made of whether or not to fight for the acquisition of the other’s shell.

Spatial awareness

Homing, meaning “any movement undertaken to reach a spatially restricted area which is known to an animal” [65], has been described in many arthropods (see the well-known case of bees), including several species of decapods [66]. Depending on the distance from which decapods are known to be able to relocate their homes (usually between 1 to 300 m) and on the habitat (subtidal, intertidal, terrestrial, etc.), homing may involve different sensorial cues and may be based on different mechanisms ranging from the direct sensorial contact with home to route-based orientation and pilotage or navigation. Examples of species relying on route-based orientation are fiddler crabs: to return to their burrow, these species integrate their path by remembering the turns taken on the outward path during foraging [67].

The decapod with the most impressive homing ability seems to be the spiny lobster *Panulirus argus* [68]. This species may undertake seasonal migrations of up to 200 km during which orientation is accomplished by the use of a magnetic sense [69]. Once displaced for over 30 km, lobsters were capable of accurate orientation towards their home location in the absence of visual or magnetic cues on the outward journey and of visual cues at the test site. Homing seems thus in this species to involve the construction of a map characterised by a detection system that gives the animal information about its current place and its home place coupled with a directional or compass sense, so that the animal can fix its path back to home.

There are other decapods with orientation abilities less spectacular than the one described in spiny lobsters but however indicative of the existence of complex cognitive maps. A remarkable example is *Thalamita crenata*, a crab dwelling muddy mangrove flats in East Africa. This species leaves its refuge, a hole in the flat, four times a day to forage to a distance of less than 5 m [70]. Field experiments showed that the crabs are faithful to a system of 2–4 holes which were visited in turn [71]. In a first set of experiments, test crabs were tagged before the start of their foraging excursions, then released 5, 20 or 50 m away from their holes. When released at a distance of 5 m, most crabs were well oriented homewards and eas-

ily returned home even when tested under the new moon or when the mangrove landscape was hidden from their view with a large vertical screen; on the contrary, they did not return home if blinded. They also showed a correct initial direction towards home from a distance of 20 m and often walked straight home, but they became dispersed from a distance of 50 m. Following the rationale of Tinbergen's study on digger wasps [72], Cannicci *et al.* [73] carried out a second set of experiments involving the use of conspicuous movable visual landmarks. Once the landmark surrounding a crab's hole had been moved 5 m away, crabs made initial navigational errors in accordance with the new position of the landmark; once the landmark were repositioned and the crabs released far from the familiar area on a similar flat, they relied only on the artificial landmark to reach the point where home should have been.

Personality

The ability to produce appropriate responses to changing conditions is clearly beneficial to an animal. However, since plasticity in its different expressions is clearly subject to a number of "evolutionary" constraints that range from sensory capabilities to cognitive structure and learning [74], it has been usually considered to be a prerogative of vertebrate taxa, with the only exception of octopuses [75]. When differences in individual behaviour are consistent across contexts or between situations, they have been characterized as "animal personalities" [76]. A key axis of animal personality is variation in the "shyness–boldness axis" [77] often simply referred to as "boldness". A bold individual will typically show high levels of exploratory behaviour in a new environment, readily investigate novel objects, and when disturbed show a "startle response" of short duration.

Recently, Briffa *et al.* [77] explored inter-individual variation in startle responses in three populations of the European hermit crabs *Pagurus bernhardus*. To elicit this response, crabs were lifted out of the water by hand, held in an inverted position for 10 s, and then replaced on the substrate with the aperture of the shell facing upward. The manipulation causes the crabs to tightly withdraw into their shell. The duration of the response, timed from when the crab was replaced on the substrate until it had re-emerged to the point where both pairs of walking legs were outside the aperture, was recorded in the field and in the laboratory in the presence or absence of predator cues. The data strongly indicate that, although hermit crabs modulate their behaviour showing a degree of plasticity between situations, this is exceeded by the extent of behavioural consistency. The observed patterns of adjustment of boldness between situations coupled with consistent individual differences of behaviour are assumed to denote, for the first time in a decapod, the presence of animal personalities with striking similarities with what observed in some vertebrate species [78].

DECAPODS AND LEGISLATION

The disparity in the way we regard the capacity of invertebrates and vertebrates to experience negative mental states might be in part fostered by current legislations [2]. For instance, the EU legislation aiming at the protection of animals used for experimental and other scientific purposes (Council Directive 86/609/EEC) [79] defines an "animal" as "any live non-human vertebrate, including free-living larval and/or reproducing larval forms". However, changes are expected [80]. In 1993, the Animals (Scientific Procedures) Act in the United Kingdom was amended to include the mollusc *Octopus vulgaris*. In 2002, the European Commission was called by the European Parliament to prepare a proposal for a revision of the Directive for some specific aspects. As part of the preparatory work for the revision, the Animal Health and Animal Welfare Panel of European Food Safety [81] produced a document that concluded with recommending an extension of the taxa to be protected by the Directive to selected invertebrates, including cephalopods and decapod crustaceans.

A public consultation on the prospected revision of the Directive in large part supported this view. To the question related to the possible inclusion in the Directive of selected invertebrate species and whether this would lead to an increase of their welfare, more than 42% of respondents (101 out of 238) stated to support the preliminary analysis of the panel. The large majority of the negative replies (totalling 32%) did not deny the need for increasing welfare of invertebrates; instead, the respondents underlined the current paucity of scientific evidence and the necessity of additional research.

Indeed, lack of specific studies is also lamented by scientists engaged in the discussion [22]. As seems clear from this review, there are few studies that examine the potential links between stress responses and pain experience. More numerous are the papers that help elucidate the presence of cognitive abilities in decapods, but they have been limited to a few taxa, and their main objective was outside the issue of "sentience".

Finally, it is worth mentioning that there is an increasing general concern about the way decapods are treated in fisheries and aquaculture. There are many current practices that may be cruel, for instance their holding in close confinement for long periods with immobilized chelae or being placed alive in boiling water to cook [22]. Research is expected to evaluate the ethic of these practices and eventually to modify them in order to respect the welfare of this vast and important group of animals.

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References

- Bateson P. Assessment of pain in animals. *Anim Behav* 1991;42:827-39.
- Sherwin CM. Can invertebrates suffer? Or how robust is argument-by-analogy? *Anim Welfare* 2001;10:S103-8.
- Stevens CW. Alternatives to the use of mammals for pain research. *Life Sci* 1992;50:901-12.
- Elwood RW, Appel M. Pain experience in hermit crabs? *Anim Behav* 2009;77:1243-46.
- Sneddon LU, Braithwaite VA, Gentle MJ. Do fishes have nociceptors? Evidence for the evolution of a vertebrate sensory system. *Proc Roy Soc B* 2003;270:1115-21.
- Loeser JD, Treede R-D. The Kyoto protocol of IASP Basic Pain Terminology. *Pain* 2008;137:473-7.
- Zimmerman M. Physiological mechanisms of pain and its treatment. *Klinische Anaesthesiol Intensivther* 1986;32:1-19.
- Broom DM. Evolution of pain. In: Lord Soulsby EJJ, Morton D (Ed.). *Pain: Its nature and management in man and animals*. Proceedings of the Royal Society of Medicine International Congress Symposium Series, vol. 246. London: Royal Society of Medicine; 2001. p. 17-25.
- Mather JA. Animal suffering: an invertebrate perspective. *J Appl Anim Welfare Sci* 2001;4:151-6.
- Office of Technology Assessment, US Congress. *Alternatives to animal use in research, testing and education*. Washington DC: US Government Printing Office; 1986.
- Eisemann CH, Jorgensen WK, Merritt DJ, Rice MJ, Cribb BW, Webb PD, Zalucki MP. Do insects feel pain? A biological view. *Experientia* 1984;40:164-7.
- Wigglesworth VB. Do insects feel pain? *Antenna* 1980;4:8-9.
- Varner G. How facts matter – on the language condition and the scope of pain in the animal kingdom. *Pain Forum* 1999;8:84-6.
- Broom DM. Cognitive ability and sentience: which aquatic animals should be protected? *Dis Aquat Org* 2007;75:99-108.
- Bradshaw EL, Bateson P. Welfare implications of culling red deer (*Cervus elaphus*). *Anim Welfare* 2000;9:3-24.
- Mason GJ, Cooper J, Clarebrough C. Frustrations of fur-farmed mink: mink may thrive in captivity but they miss having water to romp about in. *Nature* 2001;410:35-6.
- Molony V, Kent JE, McKendrick IJ. Validation of a method for assessment of acute pain in lambs. *Appl Anim Behav Sci* 2002;76:215-38.
- Smith JA, Boyd KM. *Lives in the balance: The ethics of using animals in biomedical research*. Oxford: Oxford University Press; 1991.
- Dawkins MS. Through animal eyes: what behaviour tells us. *Appl Anim Behav Sci* 2006;100:4-10.
- Mather JA, Anderson RC. Ethics and invertebrates: a cephalopod perspective. *Dis Aquat Org* 2007;75:119-29.
- Barr S, Laming PR, Dick JTA, Elwood RW. Nociception or pain in a decapod crustacean? *Anim Behav* 2008;75:745-51.
- Elwood RW, Barr S, Patterson L. Pain and stress in crustaceans? *Appl Anim Behav Sci* 2009;118:128-36.
- Stasiak KL, Maul D, French E, Hellyer P, Vanderwoude S. Species-specific assessment of pain in laboratory animals. *Con Top Lab Anim Sci* 2003;42:13-20.
- Weary DM, Neil L, Flower FC, Fraser D. Identifying and preventing pain in animals. *Appl Anim Behav Sci* 2006;100:64-76.
- Fiorito G. Is there "pain" in invertebrates? *Behav Process* 1986;12:383-8.
- Millsopp S, Laming P. Trade-offs between feeding and shock avoidance in goldfish (*Carassius auratus*). *Appl Anim Behav Sci* 2008;113:247-54.
- Kawai N, Kono R, Sugimoto S. Avoidance learning in the crayfish (*Procambarus clarkii*) depends on the predatory imminence of the unconditioned stimulus: a behavior systems approach to learning in invertebrates. *Behav Brain Res* 2004;150:229-37.
- Denti A, Dimant B, Maldonado H. Passive avoidance learning in the crab *Chasmagnathus granulatus*. *Physiol Behav* 1988;43:317-20.
- Fernandez-Duque E, Valeggia C, Maldonado H. Multitrial inhibitory avoidance learning in the crab *Chasmagnathus*. *Behav Neur Biol* 1992;57:189-97.
- Bolles RC. Species-specific defense reactions and avoidance learning. *Psychol Rev* 1970;77:32-48.
- Mather JA. Cephalopod consciousness: Behavioural evidence. *Conscious Cogn* 2008;17:37-48.
- Edelman DB, Baars BJ, Seth AK. Identifying hallmarks of consciousness in non-mammalian species. *Conscious Cogn* 2005;14:169-87.
- Baars BJ. In the theatre of consciousness: Global workspace theory: A rigorous scientific theory of consciousness. *J Consciousness Stud* 1997;4:292-309.
- Czeschlik T. Animal cognition – the phylogeny and ontogeny of cognitive abilities. *Anim Cogn* 1998;1:1-2.
- Dawkins MS. *Animal suffering: the science of animal welfare*. London: Chapman & Hall; 1992.
- Aquiloni L, Gherardi F. Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only. *J Zool London* 2008;274:171-9.
- Aquiloni L, Gherardi F. Mate assessment by size in the red swamp crayfish *Procambarus clarkii*: effects of chemical versus visual cues. *Freshw Biol* 2008;53:461-9.
- Aquiloni L, Massolo A, Gherardi F. Sex identification in female crayfish is bimodal. *Naturwissenschaften* 2009;96:103-10.
- Aquiloni L, Gherardi F. Evidence of cryptic mate choice in crayfish. *Biol Lett* 2008;4:163-5.
- Aquiloni L, Buřig M, Gherardi F. Crayfish females eavesdrop on fighting males before choosing the dominant mate. *Current Biol* 2008;18:462-3.
- Aquiloni L, Gherardi F. Crayfish females eavesdrop on fighting males and use smell and sight to recognize the identity of the winner. *Anim Behav* 2009 (in press).
- Tricarico E, Gherardi F. Shell acquisition in hermit crabs. Which tactic is more efficient? *Behav Ecol Sociobiol* 2006;60:492-500.
- Elwood RW, Stewart A. The timing of decisions during shell investigation by the hermit crab, *Pagurus bernhardus*. *Anim Behav* 1985;33:620-7.
- Jackson NW, Elwood RW. 1989. How animals make assessments: information gathering by the hermit crab, *Pagurus bernhardus*. *Anim Behav* 1989;38:951-7.
- Elwood RW, Pothanikat E, Briffa M. Honest and dishonest displays, motivational state, and subsequent decisions in hermit crab shell fights. *Anim Behav* 2006;72:853-9.
- Gherardi F. Fighting behavior in hermit crabs: the combined effect of resource-holding potential and resource value in *Pagurus longicarpus*. *Behav Ecol Sociobiol* 2006;59:500-10.
- Tricarico E, Gherardi F. Resource assessment in hermit crabs: the worth of their own shell. *Behav Ecol* 2007;18:615-20.
- Dowds BM, Elwood RW. Shell wars: assessment strategies and the timing of decisions in hermit crab fights. *Behaviour* 1983;85:1-24.
- Briffa M, Elwood RW, Dick JTA. Analyses of repeated signals during hermit crab shell fights. *Proc Roy Soc B* 1998;265:1467-74.

50. Tricarico E, Gherardi F. The past ownership of a resource affects the agonistic behavior of hermit crabs. *Behav Ecol Sociobiol* 2007;61:1945-53.
51. Briffa M, Elwood RW. Use of energy reserves in fighting hermit crabs. *Proc Roy Soc B* 2004;271:373-9.
52. Briffa M, Elwood RW. Decision rules, energy metabolism and vigour of hermit crab fights. *Proc Roy Soc B* 2001;268:1841-7.
53. Briffa M., Elwood RW. Rapid change in energetic status in fighting animals: causes and effects of strategic decisions. *Anim Behav* 2005;7:119-24.
54. Derby C D. Learning from spiny lobsters about chemosensory coding of mixtures. *Physiol Behav* 2000;69:203-9.
55. Fine-Levy JB, Girardot M-N, Derby CD, Daniel PC. Differential associative conditioning and olfactory discrimination in the spiny lobster *Panulirus argus*. *Behav Neural Biol* 1988;49:315-31.
56. Karavanich C, Atema J. Individual recognition and memory in lobster dominance. *Anim Behav* 1998;56:1553-60.
57. Hazlett BA. Predator recognition and learned irrelevance in the crayfish *Orconectes virilis*. *Ethology* 2003;109:765-80.
58. Gherardi F, Tricarico E, Atema J. Unraveling the nature of individual recognition by odor in hermit crabs. *J Chem Ecol* 2005;31:2877-96.
59. Breithaupt T, Atema J. The timing of chemical signaling with urine in dominance fights of male lobsters (*Homarus americanus*). *Behav Ecol Sociobiol* 2000;49:67-78.
60. Breithaupt T, Eger P. Urine makes the difference: chemical communication in fighting crayfish made visible. *J Exp Biol* 2002;205:1221-31.
61. Moore PA. Agonistic behavior in freshwater crayfish: the influence of intrinsic and extrinsic factors on aggressive behaviour and dominance. In: JE Duffy, M Thiel (Ed.). *Evolutionary ecology of social and sexual systems: Crustacea as model organisms*. Oxford: Oxford University Press; 2007. p. 90-114.
62. Gherardi F, Tiedemann J. Binary individual recognition in hermit crabs. *Behav Ecol Sociobiol* 2004;55:524-30.
63. Gherardi F, Tiedemann J. Chemical cues and binary individual recognition in the hermit crab *Pagurus longicarpus*. *J Zool London* 2004;263:23-9.
64. Gherardi F, Atema J. Memory of social partners in hermit crab dominance. *Ethology* 2005;111:271-85.
65. Papi F. General aspects. In: F Papi (Ed.). *Animal homing*. London: Chapman & Hall; 1992. p. 6-18.
66. Vannini M, Cannicci S. Homing behaviour and possible cognitive maps in crustacean decapods. *J Exp Mar Biol Ecol* 1995;193:67-91.
67. Zeil J, Hemmi JM. The visual ecology of fiddler crabs. *J Comp Physiol A* 2006;192:1-25.
68. Lohmann KJ, Pentcheff ND, Nevitt GA, Stetten GD, Zimmerfaust RK, Jarrard HE, Boles LC. Magnetic orientation of spiny lobsters in the ocean: experiments with undersea coil systems. *J Exp Biol* 1995;198:2041-8.
69. Boles LC, Lohmann KJ 2003. True navigation in spiny lobsters. *Nature* 2003;421:60-3.
70. Vezzosi R, Anyona D, Barbaresi S, Vannini M. Activity pattern in *Thalamita crenata* (Portunidae, Decapoda): a shaping by the tidal cycle. *Mar Behav Physiol* 1994;24:207-14.
71. Cannicci S, Anyona D, Dahdouh-Guebas F, Vannini M. Homing in the mangrove swimming crab *Thalamita crenata* (Decapoda, Portunidae). *Ethology* 1995;100:242-52.
72. Tinbergen N. *Curious naturalists*. Harmondsworth: Penguin Education; 1974.
73. Cannicci S, Fratini S, Vannini M. Short-range homing in fiddler crabs (Ocypodidae, Genus *Uca*): a homing mechanism not based on local visual landmarks. *Ethology* 1999;105:867-80.
74. Dall SRX, Houston AI, McNamara JM. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecol Lett* 2004;7:734-9.
75. Mather JA, Anderson RC. Personalities of octoposuses (*Octopus rubescens*). *J Comp Psychol* 1993;107:336-40.
76. Wilson DS, Coleman K, Clark AB, Biederman L. Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): an ecological study of a psychological trait. *J Comp Psychol* 1993;107:250-60.
77. Briffa M, Rundle SD, Fryer A. Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. *Proc Roy Soc B* 2008;275:1305-11.
78. Gosling S. From mice to men: what we can learn about personality from animal research? *Psychol Bull* 2001;127:45-86.
79. European Union. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes. *Official Journal L* 358, 18/12/1986 p. 1-28.
80. Zutphen van B. Legislation of animal use – developments in Europe. *AATEX* 2008;14:805-9.
81. European Food Safety Authority. Opinion on the “Aspects of the biology and welfare of animals used for experimental and other scientific purposes”. Adopted by the AHAW. Panel on 14 November 2005. *EFSA J* 2005;292:1-46. (http://ec.europa.eu/environment/chemicals/lab_animals/scientific_en.htm).